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Estimation of action site of L-lactic acid-ethanol-isopropyl myristate mixed system for its enhancing effect on the skin permeation of ketotifen

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Abstract

L-Lactic acid-ethanol-isopropyl myristate (IPM) mixed system markedly increased in vitro permeation of ketotifen (KT) through excised hairless rat skin. The object of this study is to verify action site(s) of the mixed system for its enhancing effect on the skin permeation. In vitro permeations of KT through full-thickness skin and stratum corneum-stripped skin from the following vehicles were in the order; silicone fluid < IPM < 1% L-lactic acid/IPM < 10% ethanol/IPM < 1% L-lactic acid-10% ethanol/IPM for both skins. Addition of L-lactic acid or ethanol increased the permeability coefficient of stratum corneum, whereas no change was evidenced for that of viable epidermis and dermis. KT permeabilities were also measured following application of these vehicles on the dermis side and concomitantly on the stratum corneum side. With IPM or 10% ethanol/IPM being used, the drug permeations from the stratum corneum side were higher than those from the dermis. KT permeations through silicone and porous polypropylene membranes, each comprising lipid and aqueous domains, were measured; ethanol exhibited an enhancing effect on permeations through both membranes. In contrast, L-lactic acid increased the KT permeation through the porous membrane only when ethanol was simultaneously used. From these findings, it was concluded as follows; (i) IPM mainly exerted effects on lipid domain of the stratum corneum; (ii) ethanol increased drug permeation through the entire region of the skin; and (iii) L-lactic acid had an effect on the aqueous domain of the stratum corneum and the layer beneath. © 1997 Elsevier Science B.V.

Keywords: Skin permeation; Penetration enhancer; Multicomponent system; Lipophilic vehicle; Action site

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1. Introduction

The permeation barrier of skin against therapeutic drugs exists in the stratum corneum and the viable epidermis/dermis. The former layer comprises two domains with different polarities, aqueous (pore) domain characterized as a hydrophilic microporous region and a lipid domain evidenced as a highly lipophilic solution-diffusion membrane (Ghanem et al., 1987; Hatanaka et al., 1995). A drug permeates through respective domains depending on its lipophilicity. A penetration enhancer also migrates to different parts of skin according to its lipophilicity. Such a selective distribution of an enhancer is associated with different types of penetration-enhancing effect (Barry, 1991; Yamashita et al., 1993); therefore, it is important to elucidate its site of action on the skin. On the other hand, the viable epidermis and dermis are regarded as representing an aqueous protein gel (Scheuplein, 1976), and permeability coefficient of various drugs via these layers is almost the same irrespective of drug polarity (Ghanem et al., 1987). Recently, it has been reported that blood flow and vascular permeability of a drug at the dermis are also involved in the permeation process in addition to the permeation barriers of the stratum corneum and the viable epidermis/dermis. Among these three barriers, stratum corneum is considered to be predominant in its contribution.

We previously reported that lipophilic multicomponent vehicles consisting of an organic acid, a lower alcohol and a fatty acid ester markedly enhanced the permeation of ketotifen (KT) through hairless rat skin (Nakamura et al., 1996). The most powerful enhancing system was verified to be a combination of L-lactic acid, ethanol and isopropyl myristate (IPM) (LEI system). However, it remains unclear where each of the components in the LEI system exerts its effect in skin layers. To elucidate this, permeation of KT was determined using full-thickness skin and stripped skin. In addition, synthetic membranes, silicone rubber and porous polypropylene membranes were also used, because they have typical lipid and pore domains, respectively (Lee et al., 1987; Hatanaka et al., 1990, 1992). Permeations of KT and these vehicle components through these membranes were also measured.

2. Experimental

2.1. Materials

Ketotifen fumarate was supplied by Sandoz Pharmaceuticals, (Tokyo, Japan). A medical grade non-reinforced Silastic sheeting (0.005 inch thick) was obtained from Dow Corning Corporation (MI, USA). Porous polypropylene membrane, Celgard 2400 (Porosity 38%, Pore diameter 0.2×0.02 μm, 25 μm thick) was obtained from Hoechst Celanese (NC, USA). Ethanol and L-lactic acid were obtained from Wako Pure Chemical Industries (Osaka, Japan). IPM was obtained from Tokyo Kasei Kogyo (Tokyo). Polydimethylsiloxane (silicone) fluid, 360 Medical Fluid (20 cs viscosity) was obtained from Daw Corning (MI, USA). Other reagents were commercially available.

2.2. Methods

2.2.1. Preparation of membranes

Epidermal membranes were excised from the abdomen of hairless rats (WBN/ILA-Ht, male, weight about 180g, Life Science Research Center, Josai University, Saitama, Japan). The full-thickness skin was excised intact. Stripped skin was obtained by stripping the stratum corneum from shaved abdominal skin (20 times) with adhesive cellophane tape (Nichiban, Tokyo, Japan) (Washitake et al., 1973). Excess fat was carefully removed from the skin using a scissors. The skin sample was used immediately after excision.

Silicone membranes were washed with tepid water and dried at room temperature overnight. Porous polypropylene membranes were used as received.

2.2.2. Permeation experiments

A side-by-side (2-chamber) diffusion cell was utilized for the in vitro permeation experiment (Okumura et al., 1990). Full-thickness skin, stripped skin, silicone or porous polypropylene membrane sample were carefully mounted between two half-cells of the 2-chamber diffusion cell set (effective area: 0.95 cm^2, cell volume: 2.5 ml). In this study, silicone fluid, IPM, 1% L-lactic

Table 1
The flux and lag time of KT through full-thickness skin and stripped skin

Vehicle	Full-thickness skin		Stripped skin	
	Flux (μg/cm^2 per h)	Lag time (h)	Flux (μg/cm^2 per h)	Lag time (h)
Silicone	0.3 ± 0.1	7.5 ± 2.0	25.3 ± 8.6	1.5 ± 1.2
IPM	2.5 ± 0.2	5.0 ± 0.3	183.6 ± 44.8	1.3 ± 0.3
Ll system	18.5 ± 1.4	2.9 ± 0.1	259.7 ± 139.3	1.3 ± 0.4
El system	2044.4 ± 121.8	2.2 ± 0.3	4064.9 ± 579.0	1.6 ± 0.2
LEI system	2785.2 ± 254.2	0.6 ± 0.1	5326.8 ± 612.6	1.1 ± 0.2

acid/IPM (LI system), 10% ethanol/IPM (EI system) and 1% L-lactic acid-10% ethanol/IPM (LEI system) were employed for lipophilic vehicles. Ketotifen fumarate suspension (ca. twice solubility) in each solvent system was usually applied on the stratum corneum side of 2-chamber diffusion cell, and the dermis side was filled with distilled water. In case of vehicle application from the dermis side, ketotifen fumarate-suspended vehicle was placed in the dermis side compartment and distilled water in the stratum corneum side. The permeation experiments were performed at 37°C. Sample solution was withdrawn periodically from the receiver side chamber, and the same volume of distilled water was added after sampling. When (pseudo) steady-state flux was determined, the donor vehicle was changed periodically to avoid marked decrease in thermodynamic activity of KT and vehicle components in the system throughout the experiment.

2.3. Analytical method

KT was determined by HPLC. Sample containing KT was added to acetonitrile solution containing ethyl *p*-hydroxylbenzoate as an internal standard. After centrifugation of the mixed solution, the supernatant was injected into HPLC composed of a pump system (LC-6A, Shimadzu Seisakusho, Kyoto), a UV detector (SPD-6A, Shimadzu Seisakusho), Chromatopack (CR-3A, Shimadzu Seisakusho), and a reverse phase column (Ultron N-C18; 150 × 4.6mm i.d., Shinwa Kako, Kyoto). The mobile phase was 0.1% phosphoric acid:acetonitrile (70:30), the flow rate was 0.8 ml/min, and detection was conducted at UV-295 nm.

Ethanol was measured by GC (GC-8A, Shimadzu Seisakusho). The conditions were: injection volume, 1 μl; column, Gaskuropack 54 (60/80 mesh, GL Science, Tokyo); temperatures for column, injection port and detector, 160, 200 and 200°C, respectively; carrier gas, N_2; carrier pressure, 1 kg/cm^2. A flame ionization detector (FID) was used, and the absolute calibration method was applied.

L-Lactic acid was assayed by GC. The conditions were: column, Thermon-3000 (support Shincarbon A, 60/80 mesh, GL Science); temperatures for column, injection port and detector, 140, 180 and 180°C, respectively; carrier gas, N_2; carrier pressure, 0.5 kg/cm^2. FID and the absolute calibration method were used.

IPM was determined by GC, and the conditions were: column, Silicone-OV101 (support Chromosorb G (HP), 100/120 mesh, Shinwa Kako); temperatures for column, injection port and detector, 200, 230 and 230°C, respectively; carrier gas, N_2; carrier pressure, 1 kg/cm^2. FID and the absolute calibration method were used.

3. Results and discussion

3.1. Comparison of KT permeation through full-thickness skin and stripped skin

Fluxes, lag times and permeability coefficients of KT and vehicle components through full-thickness and stripped skin were measured to elucidate which layer of the skin membrane (stratum corneum or viable epidermis/dermis) was affected by the lipophilic multicomponent system being

Table 2
Permeability coefficient of each layer for KT permeation from various vehicles

Vehicle	KF solubility (mg/ml)	P ($\times 10^{-4}$ cm/s)		
		Full-thickness skin	Stripped skin	S.C.[a]
Silicone	0.00841	0.09 ± 0.02	8.36 ± 2.85	0.09
IPM	0.105	0.07 ± 0.01	4.86 ± 1.19	0.07
Ll system	0.106	0.48 ± 0.04	6.80 ± 3.65	0.52
El system	0.855	6.64 ± 0.40	13.21 ± 0.19	13.35
LEI system	2.22	3.49 ± 0.32	6.67 ± 0.77	7.33

[a]; Stratum corneum.

composed of L-lactic acid, ethanol and IPM. Silicone fluid, an inert liquid against the skin barrier, was selected as a control vehicle.

Table 1 summarizes the fluxes and lag times of KT permeation through both skins. The flux via full-thickness skin from the following vehicles was in the order of, silicone < IPM < LI system < EI system < LEI system while similar rank order was observed in the stripped skin. IPM alone increased the KT fluxes 7–8 times compared to those by silicone fluid through full-thickness skin and stripped skin. When L-lactic acid was added to IPM (LI system), the drug permeation was enhanced 7.5 times for full-thickness skin and about 1.5 times for stripped skin. With addition of ethanol into IPM (EI system), the effect was enhanced about 800 and 20 times for full-thickness and stripped skins, respectively. When ethanol was included in the LI system (LEI system), the permeation was increased 150 and 20 times for both skin membranes, respectively. Addition of L-lactic acid to the EI system (LEI system) exhibited 1.3 times higher KT permeation through both skins than the EI system. It is verified from these results that L-lactic acid, ethanol and IPM exert enhancing effect on not only the stratum corneum but also the viable epidermis/dermis.

The order of lag times of KT permeation through full-thickness skin placed LEI system < EI system < LI system < IPM < silicone fluid, that through stripped skin was similar. These lag times include a period when the enhancer migrates into skin to exhibit its action as well as time for reaching steady-state permeation of KT. With IPM, LI system, EI system, or LEI system being applied on the full-thickness skin, the lag time was decreased presumably due to increase of KT diffusion in the stratum corneum.

Table 2 shows the permeability coefficients of KT across each layer from various vehicles. The permeability coefficient across stratum corneum was calculated from the permeabilities across full-thickness skin and stripped skin. Ratio of the coefficient (stratum corneum/viable epidermis and dermis) was 1/100, 1/70, 1/10, 1 and 1 for silicone fluid, IPM, LI, EI and LEI system, respectively. In the vehicles containing ethanol, the permeability coefficients were markedly increased. IPM hardly changed the coefficient of stratum corneum relative to silicone fluid, whereas that for LI, EI, and LEI systems was increased about 6, 150, and 80 times, respectively. On the other hand, alteration was hardly evidenced for the permeability coefficient in viable epidermis/dermis. Given all these findings, IPM mainly affected the stratum corneum, while L-lactic acid and ethanol had an effect both on the stratum corneum and the viable epidermis/dermis.

Tables 1 and 2 delineate two interesting points: (i) the KT fluxes through the stripped skin from the drug suspended IPM, LI, EI or LEI system were higher than that from the silicone fluid; however, the permeability coefficients were almost similar to each other; and (ii) the rank order of KT solubility in vehicles was equivalent to that of KT flux through the stripped skin. These results verified that enhancing effects by the lipophilic systems were related to an increase of KT solubility in the systems. Under assumptions that skin is

Table 3
The flux of ethanol and L-lactic acid through hairless rat skin

Vehicle	Ethanol (mg/cm^2 per h)		L-lactic acid (mg/cm^2 per h)	
	F.T. skin[a]	Stripped skin	F.T. skin[a]	Stripped skin
Ll system			0.76 ± 0.07	1.45 ± 0.18
El system	40.0 ± 4.81	54.20 ± 3.98		
LEl system	49.0 ± 3.35	57.09 ± 1.12	4.88 ± 0.16	5.20 ± 0.11

[a]; Full-thickness skin.

homogeneous and behaves like a solution-diffusion membrane free from any interaction with vehicle, the partition coefficient of a penetrant is decreased at the same rate with increasing drug solubility in the vehicle, resulting in no alteration in the drug flux (Higuchi, 1960; Higuchi and Roche, 1977). However, increase in the drug solubility is responsible for the increase of drug flux in either of the following cases; when an enhancer acts as a cosolvent after migration into skin (Berner et al., 1989), when there is a domain filled with a vehicle (i.e. pore or aqueous domain), or when a solvent penetrates across skin with a drug (solvent drag) (Karino et al., 1982; Sato et al., 1988). It is conceivable that KT flux through the viable epidermis/dermis might be increased by these modes of action.

Table 3 shows the fluxes of vehicle components through hairless rat skin. IPM penetration was not detected for all vehicles, but it is more likely that IPM could distribute into the stratum corneum because of its relatively low molecular weight and highly lipophilic nature (Goldberg-Cettina et al., 1995). Ethanol permeated through the full-thickness and the stripped skins in a similar degree (each permeability coefficient $= 2 \times 10^{-4}$ cm/s). Although the stratum corneum is a great barrier to permeation of most compounds, its diffusion resistance was markedly reduced by IPM and ethanol. The diffusion through the viable epidermis and dermis is a decisive factor of the ethanol flux because their thickness (720 μm, Kobayashi et al., 1994) is much larger than that of the stratum corneum (15 μm, Sato et al., 1991). The ethanol permeation was not affected by either presence or absence of L-lactic acid.

The flux of L-lactic acid through full-thickness skin was increased at 6.5-fold by addition of ethanol. The L-lactic acid flux from LI system was 2-fold increased by elimination of the stratum corneum whereas that from LEI system was not changed by the stripping procedure. These results show that the stratum corneum and viable epidermis/dermis possess similar permeation resistance for permeation of L-lactic acid in the absence of ethanol. With concomitant use of L-lactic acid with ethanol, the stratum corneum rarely acted as a barrier to the permeation of both L-lactic acid and ethanol. It was conceivable that ethanol might enhance the acid permeation both through the stratum corneum and the layer beneath, and thereby the increased L-lactic acid permeation might then promote the KT flux. When the LEI system was used, permeability coefficients of L-lactic acid, ethanol, and KT were almost the same (1.7×10^{-4}, 2×10^{-4}, and 3.5×10^{-4} cm/s, respectively), despite the significant difference in their molecular weights and solubility parameters (90.08 and 14.8, 46.07 and 12.6, and 309.43 Da and 11.53 $(cal/cm^3)^{1/2}$). These findings were consistent with a previous work by Ghanem et al. (1987), who reported that the permeability coefficient of viable epidermis and dermis for various solutes was the same when permeation resistance of the stratum corneum was negligible.

It was suggested from the above results that increase of the drug solubility in the membrane was involved in the enhancing effect of the LEI system on the KT permeation through the viable epidermis and dermis; furthermore, increase of partitioning and/or diffusivity of the drug in the stratum corneum layer might also contribute to the promoting effect of LEI system.

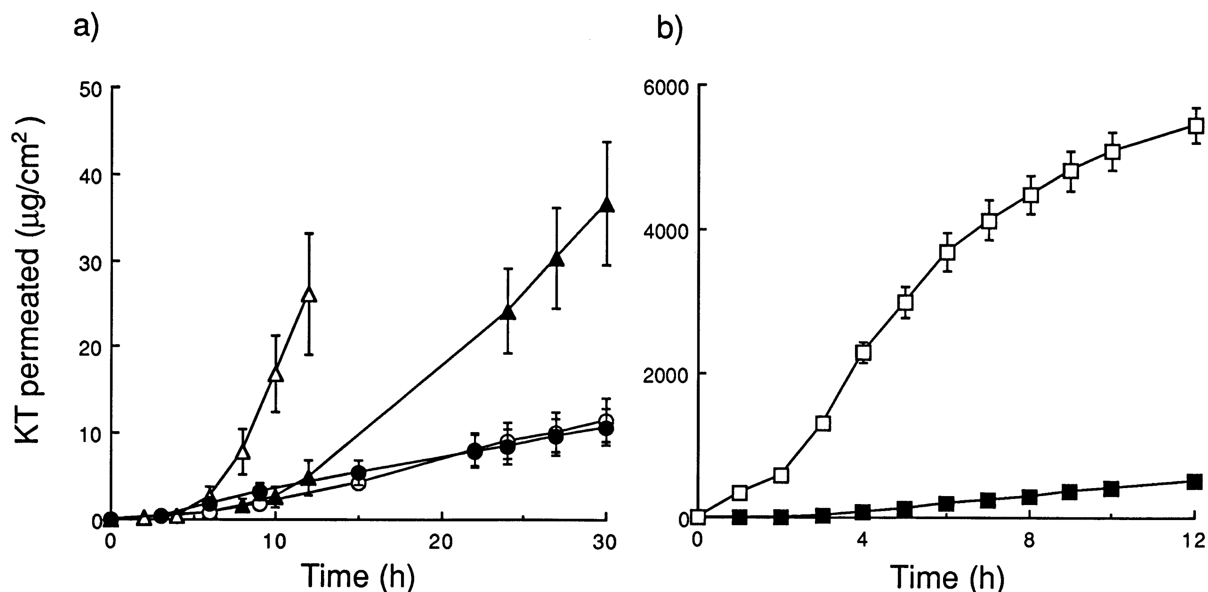

Fig. 1. In vitro permeation of KT through hairless rat skin from stratum corneum to dermis side and from dermis to stratum corneum side; (open symbol) stratum corneum side to dermis side, (closed symbol) dermis side to stratum corneum side (a) Silicone fluid (○,●) and IPM (△,▲), (b) EI system (□,■). Each value represents the mean ± S.E. ($n = 3$–9).

3.2. Comparison of KT permeations from stratum corneum and dermis as the donor sides

Stratum corneum is a highly lipophilic membrane, whereas the dermis is hydrophilic. Following application of the vehicle onto either the stratum corneum or dermis, a difference was seen in the migrated amount of vehicle components into skin and the enhancing effect (Morimoto et al., 1986). This experimental methodology is useful to locate the site of action of a vehicle component in the skin and to monitor the distribution pattern of a vehicle into the skin. LEI system consists of three components having different lipophilicities. IPM is highly lipophilic, resulting in more distribution to the stratum corneum relative to the dermis. Ethanol easily penetrates into both layers, and L-lactic acid is preferentially distributed to hydrophilic domain of the skin. To evaluate the migration profiles of enhancer components depending on their effects, the enhancer system was applied to either the stratum corneum side or the dermis side, and the flux ratios of KT or ethanol permeation from stratum corneum to dermis against dermis to stratum corneum (S/D ratio) were compared.

Figs. 1 and 2 illustrate permeation profiles of KT and ethanol, respectively, from the stratum corneum or dermis side to another side. When silicone fluid, KT fluxes from both sides were almost the same (S/D ratio = 1, while following application of IPM, the S/D ratio was ≈ 2. KT permeation from the dermis side was equal to that for silicone fluid over 12 h, besides being three times higher than silicone fluid thereafter. These findings suggested that IPM exhibited a remarkable enhancing effect on the stratum corneum, despite slow penetration of IPM through viable epidermis and dermis. On the EI system, S/D ratio was about 50, being higher than those of IPM application.

Ethanol permeation from stratum corneum side was also evidenced to be higher than that from dermis side. IPM in contact with the stratum corneum enhanced both the KT and the ethanol permeations; however, S/D ratio of KT was

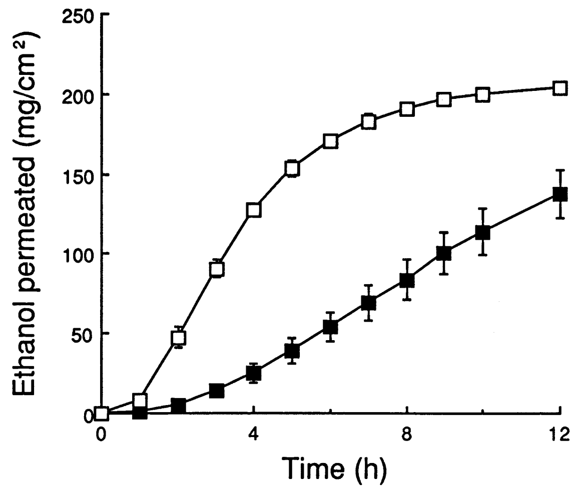

Fig. 2. In vitro permeation of ethanol through hairless rat skin from stratum corneum to dermis side and from dermis to stratum corneum side; (open symbol) stratum corneum side to dermis side, (closed symbol) dermis side to stratum corneum side. Each value represents the mean ± S.E. ($n = 3$–6).

greater than that of ethanol. It is likely that KT permeability was enhanced not only by IPM, but also ethanol characterized with increased permeation by IPM.

3.3. Comparison of permeations through the artificial membranes

Enhancing effect of various vehicles was measured on the KT permeability through silicone membrane and porous polypropylene membrane to estimate which domain, aqueous or lipid, is more contributory to the effect of LEI system.

Fig. 3 illustrates the permeation profiles of KT through silicone and porous polypropylene membranes. By addition of ethanol, the KT permeation through silicone membrane was more increased than with IPM, but it was slightly decreased by addition of L-lactic acid in comparison to the corresponding systems without L-lactic acid. By addition of ethanol, the KT permeation through porous polypropylene membrane was also increased compared with IPM, but, it was not decreased by addition of L-lactic acid in comparison to EI system. KT fluxes from IPM, LI system, EI system, and LEI system were 18, 7, 183, and 335 $\mu g/cm^2$ per h, respectively.

Permeation of vehicle components through the artificial membranes is shown in Fig. 4. Ethanol permeation through silicone membrane was almost the same irrespective of whether EI or LEI

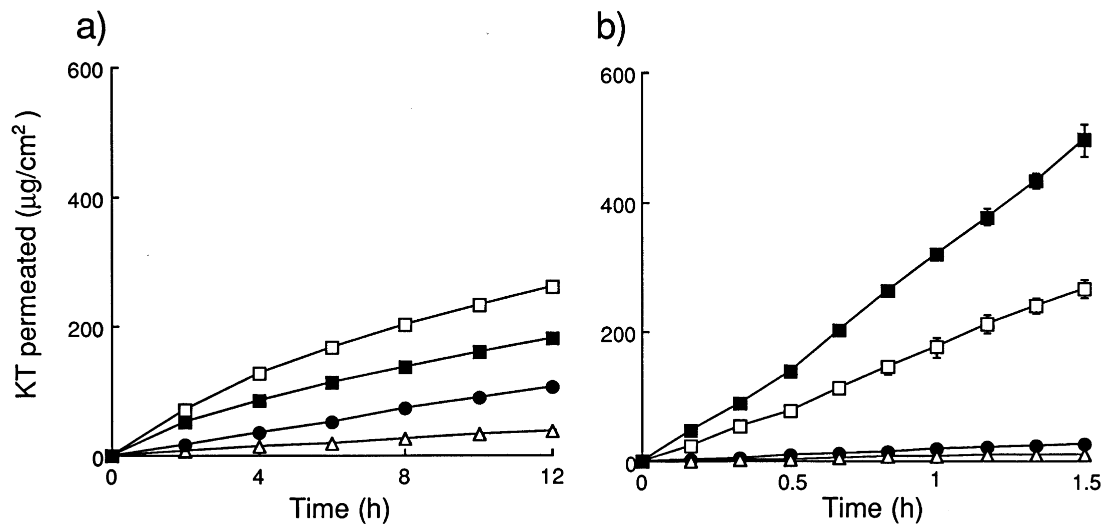

Fig. 3. In vitro permeation of KT through artificial membranes: (a) Silicone membrane; (b) Porous polypropylene membrane; ●: IPM, △: LI system, □: EI system ■: LEI system. Each value represents the mean ± S.E. ($n = 3$).

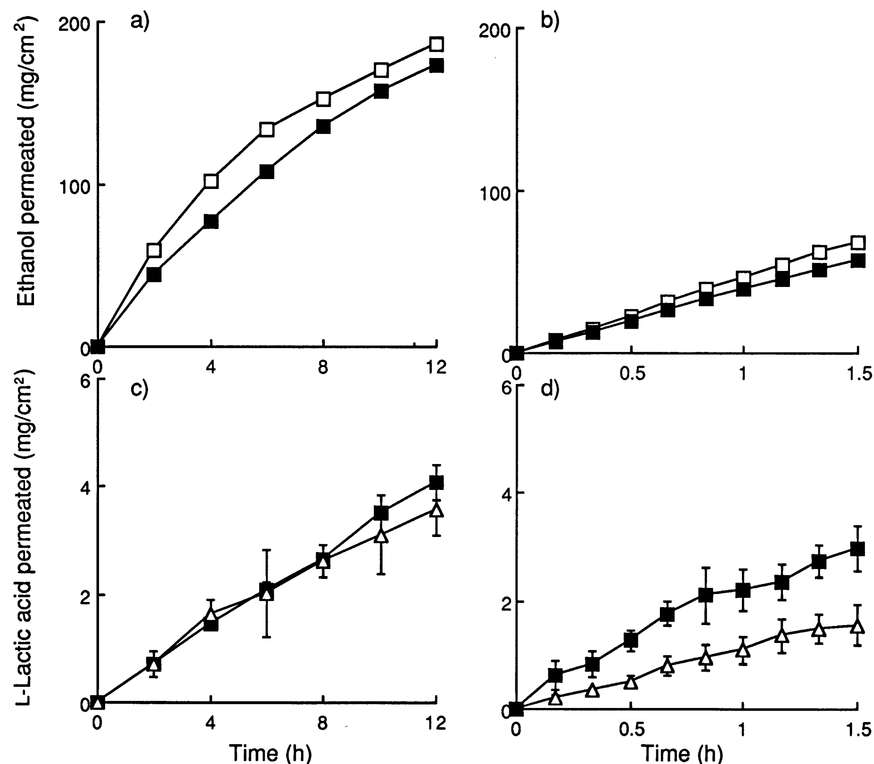

Fig. 4. In vitro permeation of L-lactic acid and ethanol through artificial membranes (a) Silicone membrane—ethanol, (b) Porous polypropylene membrane—ethanol, (c) Silicone membrane—L-lactic acid, d) Porous polypropylene membrane—L-lactic acid; △: LI system, □: EI system, ■: LEI system. Each value represents the mean ± S.E. ($n = 3$).

systems were employed, and no difference was evidenced in L-lactic acid permeations from LI or LEI systems. The ethanol permeation through porous polypropylene membranes from EI system was similar to that from LEI systems, results were same with silicone membrane, whereas L-lactic acid permeation was increased by addition of ethanol in comparison to LI system. L-Lactic acid permeation was stimulated 2-fold by ethanol. L-Lactic acid permeation through porous polypropylene membrane was higher than that through silicone membrane because of less migration of L-lactic acid into the silicone membrane due to its high polarity.

Ethanol had a promoting effect on the KT permeation through both silicone and porous polypropylene membranes, while L-lactic acid exerted the effect only on the porous polypropylene membrane. The effect of L-lactic acid was evidenced only when ethanol was applied to LI system. These results suggested that action sites of ethanol were both aqueous and lipid domains into skin and that of L-lactic acid was only an aqueous domain.

The enhancing effects of ethanol and L-lactic acid on KT permeation through these artificial membranes were compared with those across the skin. By addition of ethanol into IPM, the permeation was increased by about 800, 2, and 10 times across skin, silicone and porous polypropylene membranes, respectively. Our previous study indicated that high ethanol flux resulted in high KT flux (Nakamura et al., 1996). Although high ethanol permeation was obtained in the artificial membranes as was obtained with the skin, enhancing effect of ethanol on the permeation of KT across artificial membranes was markedly lower than those across the skin.

By addition of L-lactic acid into IPM, KT permeation through full-thickness skin was improved 7.5-times, unlike the absence of such an improvement across artificial membrane. L-Lactic acid flux through epidermal, silicone, and porous polypropylene membranes from LI system were 0.76, 0.25, and 1.09 mg/cm^2 per h, and the counterparts from LEI system were 4.88, 0.33, and 1.89 mg/cm^2 per h, respectively. The skin permeability of L-lactic acid following addition of only the acid into IPM was lower than that across the porous polypropylene membranes. With coexistence of L-lactic acid and ethanol, the skin permeability of the acid was higher than that across artificial membranes.

Possible enhancing mechanisms on the artificial membrane permeation comprise both solvent drag effect and increased membrane distribution of penetrants; furthermore structural or morphological change in stratum corneum lipid is also involved in the enhancing mechanism on the skin permeation. These differences may account for higher promoting effect by ethanol on the skin permeations of KT and L-lactic acid than those on the artificial membranes.

In conclusion, IPM mainly affected lipid domain of the stratum corneum, while ethanol increased the drug permeation through the whole region of the skin, besides L-lactic acid having an effect on the aqueous domain of the stratum corneum and the layer beneath, under coexistence of ethanol. Concomitant application of such enhancers having different action sites will enable one to develop several possible topical delivery systems in the future.

References

Barry, B.W., 1991. Lipid-protein-partitioning theory of skin penetration enhancement. J. Control. Release 15, 237–248.

Berner, B., Mazzenga, G.C., Otte, J.H., Steffens, R.J., Juang, R.-H., Ebert, C.D., 1989. Ethanol:water mutually enhanced transdermal therapeutic system II: Skin permeation of ethanol and nitroglycerin. J. Pharm. Sci 78, 402–407.

Ghanem, A.-H., Mahmoud, H., Higuchi, W.I., Rohr, U.D., Borsadia, S., Liu, P., Fox, J.L., Good, W.R., 1987. The effects of ethanol on the transport of β-estradiol and other permeants in hairless mouse skin. II. A new quantitative approach. J. Control. Release 6, 75–83.

Goldberg-Cettina, M., Liu, P., Nightingale, J., Kurihara-Bergstrom, T., 1995. Enhanced transdermal delivery of estradiol in vitro using binary vehicles of isopropyl myristate and short-chain alkanols. Int. J. Pharm. 114, 237–245.

Hatanaka, T., Inuma, M., Sugibayashi, K., Morimoto, Y., 1990. Prediction of skin permeability of drugs. I: Comparison with artificial membrane. Chem. Pharm. Bull. 38, 3452–3459.

Hatanaka, T., Inuma, M., Sugibayashi, K., Morimoto, Y., 1992. Prediction of skin permeability of drugs. II. Development of composite membrane as a skin alternative. Int. J. Pharm. 79, 21–28.

Hatanaka, T., Katayama, K., Koizumi, T., Sugibayashi, K., Morimoto, Y., 1995. Time-dependent percutaneous absorption enhancing effect of ethanol. J. Control. Rel. 33, 423–428.

Higuchi, T., 1960. Physical chemical analysis of percutaneous absorption process from creams and ointments. J. Soc. Cosmetic Chem. 11, 85–97.

Higuchi, T., Roche, B. (Eds.), 1977. Design of Biopharmaceutical Properties through Prodrugs and Analogs. American Pharmaceutical Association, Washington, DC, 409 pp.

Karino, A., Hayashi, M., Horie, T., Awazu, S., Minami, H., Hanano, M., 1982. Solvent drag effect in drug intestinal absorption. I. Studies on drug and D_2O absorption clearances. J. Pharmacobio-Dyn. 5, 410–417.

Kobayashi, D., Matsuzawa, T., Sugibayashi, K., Morimoto, Y., Kimura, M., 1994. Analysis of the combined effect of l-menthol and ethanol as skin permeation enhancers based on a two-layer skin model. Pharm. Res. 11, 96–103.

Lee, S.J., Kurihara-Bergstrom, T., Kim, S.W., 1987. Ion-paired drug diffusion through polymer membranes. Int. J. Pharm. 47, 59–73.

Morimoto, Y., Sugibayashi, K., Hosoya, K., Higuchi, W.I., 1986. Penetration enhancing effect of Azone on the transport of 5-fluorouracil across the hairless rat skin. Int. J. Pharm. 32, 31–38.

Nakamura, H., Yanee, P., Hayashi, T., Sugibayashi, K., Morimoto, Y., 1996. Effect of lipophilic multicomponent system on the skin permeation of ketotifen fumarate. Int. J. Pharm. 141, 71–80.

Okumura, M., Nakamori, Y., Yoshida, Y., Niwa, H., Sugibayashi, K., Morimoto, Y., 1990. Effect of monoglycerides on the percutaneous absorption of papaverine hydrochloride. Drug Des. Del. 6, 137–148.

Sato, K., Sugibayashi, K., Morimoto, Y., 1988. Effect and mode of action of aliphatic esters on the in vitro skin permeation of nicorandil. Int. J. Pharm. 43, 31–40.

Sato, K., Sugibayashi, K., Morimoto, Y., 1991. Species differences in percutaneous absorption of nicorandil. J. Pharm. Sci. 80, 104–107.

Scheuplein, R.J., 1976. Mechanism of percutaneous absorption. II. Transient diffusion and the relative importance of various routes of skin permeation. J. Invest. Dermatol. 48, 79–88.

Yamashita, F., Yoshioka, T., Koyama, Y., Okamoto, H., Sezaki, H., Hashida, M., 1993. Analysis of skin penetration enhancement based on a two-layer skin diffusion model with polar and nonpolar routes the stratum corneum: Dose-dependent effect of 1-geranylazacycloheptan-2-one on drugs with different lipophilicities. Biol. Pharm. Bull. 16, 690–697.

Washitake, M., Yajima, T., Anmo, T., Arita, T., Hori, R., 1973. Studies on percutaneous absorption of drugs. III. Percutaneous absorption of drugs through damaged skin. Chem. Pharm. Bull. 21, 2444.